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Cytological Analyses of 19 Taxa in *Globba* (Zingiberaceae)

ATSUKO TAKANO

Botanical Gardens, Faculty of Science, Osaka City University,
*2000 Kisaichi, Katano, Osaka 576-0004, Japan.**

Somatic chromosome numbers in 19 taxa of sects. *Ceratanthera* and *Globba* in *Globba* (Zingiberaceae) were determined to be 32 or 48. Pollen fertility of some species was also studied. Pollen fertility of plants with 2n = 32 was relatively high, while fertility of taxa with 2n = 48 was relatively low. Results of this and former cytological studies indicate that the basic chromosome number of sects. *Ceratanthera* and *Globba* is 16.

Key words: Chromosome numbers,*Globba*, Sumatra, Zingiberaceae

Globba (Zingiberaceae), a genus comprising about 100 species, is distributed in Southeast Asia. Four sections are recognized in the genus, *Ceratanthera*, *Globba*, *Haplanthera* and *Nudae* (Schumann 1904, Larsen 1972). Cytological studies in the genus (e.g., Chakravorti 1948, Sharma & Bhattacharyya 1959, Ramachandran 1969, Mehra & Sachdeva 1979, Okada 1984) have revealed a variety of chromosome numbers: 2n = 22, 24, 28, 32, 34, 44, 48, 64, 80 and 96 (Table 1). The basic chromosome number of *Globba* has not been determined, but various numbers were proposed, e. g., $x = 12$ (Raghavan & Venkatasubban 1943), 11 and 12 (Ramachandran 1969), 12 and 16 (Mahanty 1970) and 8 (Lim 1972). Different basic chromosome numbers for the different sections have also been proposed. Larsen (1972) studied 13 species in the four sections in Thailand and proposed a base number for each: x = 6 and 7 for sect. *Haplanthera*, x = 8 for sects. *Ceratanthera* and *Globba*, and x = 17 for sect. *Nudae*. Newman (1988) proposed x = 11, 12, 14 and 16 for sect. *Haplanthera*, x = 16 for sects. *Ceratanthera* and *Globba*, and x = 17 for sect. *Nudae*. There is little agreement on a the chromosome number of *Globba*.

Sumatra, where 16 species occur, is one of the centers for diversification in *Globba*. The only cytological study of *Globba* in Sumatra (Okada 1984) reported a chromosome number for *G. variabilis* as 2n = 48.

To clarify the cytological confusion and to contribute to an understanding of the basic chromosome number of *Globba*, chromosome counts and pollen fertility of various species of *Globba*, mainly from Sumatra, are reported here.

Materials and Methods

I studied 19 taxa in sects. *Ceratanthera* and

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* Present address: Museum of Nature and Human Activities, Hyogo, 6 Yayoigaoka, Sanda, Hyogo 669-1546, Japan.

TABLE 1. Published chromosome numbers in *Globba* (modified from Newman 1988).

Section Species	n	2n	Reference
Haplanthera			
Globba aphanantha K. Larsen	–	28	Larsen, 1972
G. clarkei Baker	12	24	Larsen, 1972
G. hookeri C. B. Cl.	–	22	Sharma & Bhattacharyya, 1959
	12	–	Mehra & Sachdeva, 1979
G. ophioglossa C. H. Wright	11	22	Ramachandran, 1968
G. racemosa Smith	–	24	Sharma & Bhattacharyya, 1959
G. racemosa Smith	16		Sarkar *& al.*, 1974
G. racemosa Smith	–	24	Chen *& al.*, 1984
Ceratanthera			
G. albiflora Ridl.			
var. *albiflora*	16	32	Lim, 1972
var. *aurea* Holttum	16	32	Lim, 1972
G. brachyanthera K. Schum.	–	32	Newman, 1988
G. fasciata Ridl.	–	32	Newman, 1988
G. leucantha Miq			
var. *peninsularis* Holttum	16	–	Lim, 1972
G. pendula Roxb.			
ssp. *pendula*	–	32, 48	Larsen, 1972; Lim, 1972
ssp. *pendula* var. *elegans* (Ridl.) Holttum	16	32	Lim, 1972
ssp. *montana* (Ridl.) S. N. Lim	16	32	Lim, 1972
Globba			
G. atrosanguinea Teysm. & Binn.	24	48	Mahanty, 1970; Newman, 1988
G. cernua Baker			
ssp. *cernua*	16, 24	32, 48	Lim, 1972
ssp. *crocea* S. N. Lim	16, 24	32, 48	Lim, 1972
ssp. *porphyria* S. N. Lim	16	32	Lim, 1972
G. curtisii Holttum	24	48	Lim, 1972
G. fragilis S. N. Lim	16	32	Lim, 1972
G. garrettii Kerr	–	32, 48	Larsen, 1972
G. holttumii S. N. Lim			
ssp. *holttumii* S. N. Lim	24	48	Lim, 1972
ssp. *aurea* S. N. Lim	24	48	Lim, 1972
G. × *intermedia* S. N. Lim	24	48	Lim, 1972
G. kerrii Craib	–	32	Larsen, 1972
G. laeta K. Larsen	–	32	Larsen, 1972
G. marantina L.		24, 44, 48 64, 80, 96	*
G. nisbethiana Craib	–	ca. 32	Larsen, 1972
G. obscura K. Larsen		32	Larsen, 1972
G. patens Miq			
var. *patens*	16, 24	32, 48	Lim, 1972
var. *costulata* S. N. Lim	16	32	Lim, 1972
G. propinqua Ridl.	–	ca. 32	Newman, 1988
G. purpurascens Craib	–	32	Larsen, 1972
G. reflexa Craib		32	Larsen, 1972
G. schomburgkii Hook. f.	–	48, ca. 64	Bisson *& al.*, 1968; Larsen, 1972
G. unifolia Ridl.			
var. *sessilifolia* Holttum		32	Lim, 1972
G. variabilis Ridl.			
ssp. *variabilis*	16	32	Lim, 1972
ssp. *pusilla* S. N. Lim	16	48	Lim, 1972
G. variabilis Ridl.	–	48	Okada, 1984
G. winitii C. H. Wright	–	32, 48	Bisson *& al.*, 1968; Larsen, 1972
Nudae			
G. nuda K. Larsen	17	34	Larsen, 1972

* Raghavan & Venkatasubban 1943, Sharma & Bhattacharyya 1959, Bhattacharyya 1968, Ramachandran 1969, Mahanty 1970, Lim 1972, Newman 1988.

Globba from Sumatra and Java (Indonesia), Malaysia, the Philippines and Thailand (Table 2).

Somatic chromosomes were observed using root tips. Root tips were incubated in 0.025 % aqueous colchicine solution for 2 hours at 18 °C, and fixed in 45 % acetic acid for more than 15 minutes at 4 °C, then macerated in 1N HCl for 60 seconds at 60 °C. Meristem tissue was stained on a slide glass with 2.0 % aceto-orcein for 5 minutes, then squashed.

The shape of the chromosomes was described based on the position of the centromere following Levan *et al.* (1964).

Pollen grains were stained with lacto-phenol-cotton-blue solution for 10 minutes to determine fertility. At least 100 (usually 250 - 400) pollen grains per flower were observed. Darkly stained and globose pollen grains were judged to be fertile; poorly stained, shriveled pollen was assumed to be sterile. Pollen fertility was determined as the ratio of the number of fertile grains to total grains. In some plants with 2n = 48, moderately stained, globose grains or darkly stained, almost globose grains were observed frequently. They were counted as fertile.

Results

Chromosome numbers of the species of sect. Ceratanthera

The chromosome numbers of the six species and two varieties of *G. leucantha* in sect. *Ceratanthera* examined in this study are listed in Table 2. The somatic chromosome number was found to be 32 in *Globba campsophylla*, *G. franciscii*, *G. leucantha* var. *flavidula*, *G. pendula* and *G. platystachya*, and 48 in *G. maculata*. *Globba leucantha* var. *bicolor* and *G. paniculata* had cytotypes of 2n = 32 and 2n = 48 (Figs. 1-6). This is the first report of chromosome numbers for six species: *G. campsophylla*, *G. franciscii*, *G. maculata*, *G. paniculata* and *G. platystachya*. Lim (1972) reported 2n = 32 for *G. leucantha* var. *peninsularis*. This is the first report for *G. leucantha* var. *bicolor* and var. *flavidula*. *Globba pendula*, which is widely distributed from India to the Malay Peninsula, Borneo and Sumatra, was reported to have 32 and 48 chromosomes on the Malay Peninsula (Larsen 1972; Lim 1972). However, I found only 2n = 32 in plants from Sumatra.

The length of the chromosomes varies gradually in the genome of *Globba*, ranging from 0.7 to 3.6 μm among the species studied. In all species, the longest chromosome was never more than three times the length of the shortest one. Metacentric and submetacentric chromosomes were dominant. In the plants of *G. paniculata* with 2n = 32, two metacentric chromosomes had a satellite at the distal end of the long arm (Figs. 3, 4). In plants with 2n = 48, three chromosomes had a satellite: two of them are metacentric with a satellite at the distal end of the long arm; the other is submetacentric with a satellite at the distal end of the short arm (Figs. 5, 6). In *G. franciscii* (2n = 32), two submetacentric chromosomes with a satellite at the distal end of the long arm were observed (Figs. 7, 8).

Chromosome numbers of the species of sect. Globba

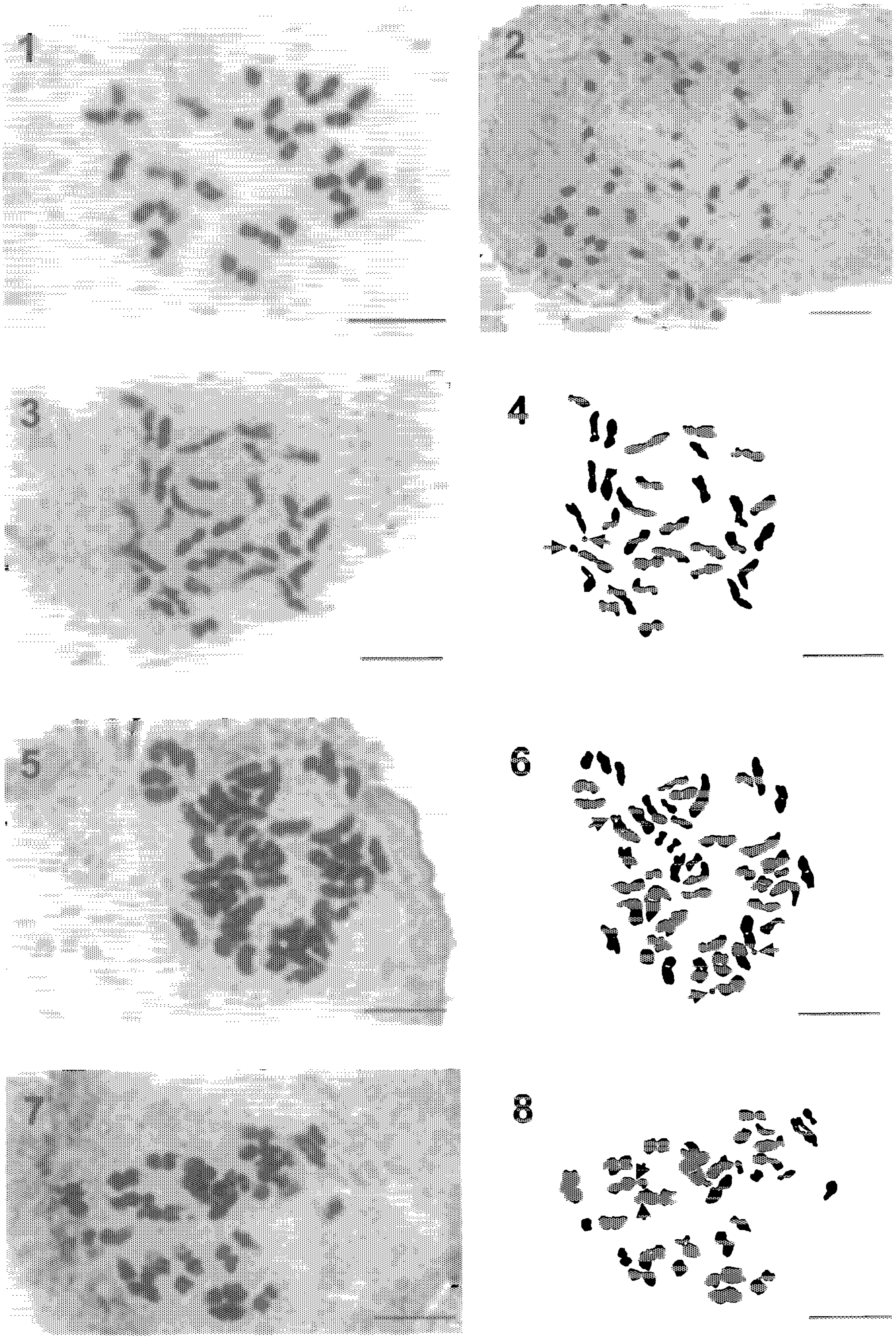
The chromosome numbers of the eleven species studied in sect. *Globba* are listed in Table 2. The somatic chromosome number was found to be 32 in *G. fecunda* and in an unidentified species of *Globba* (sp. 1) and 48 in *G. acehensis*, *G. albobracteata*, *G. atrosanguinea* var. *atrosanguinea*, *G. aurantiaca*, *G. cernua*, *G. flavibracteata*, *G. patens* and *G. talangensis*. *Globba multifolia* had cytotypes of 2n = 32 and 48 (Figs. 9, 10). This is the first report of the

TABLE 2. Chromosome numbers and collection data of the species of *Globba*.

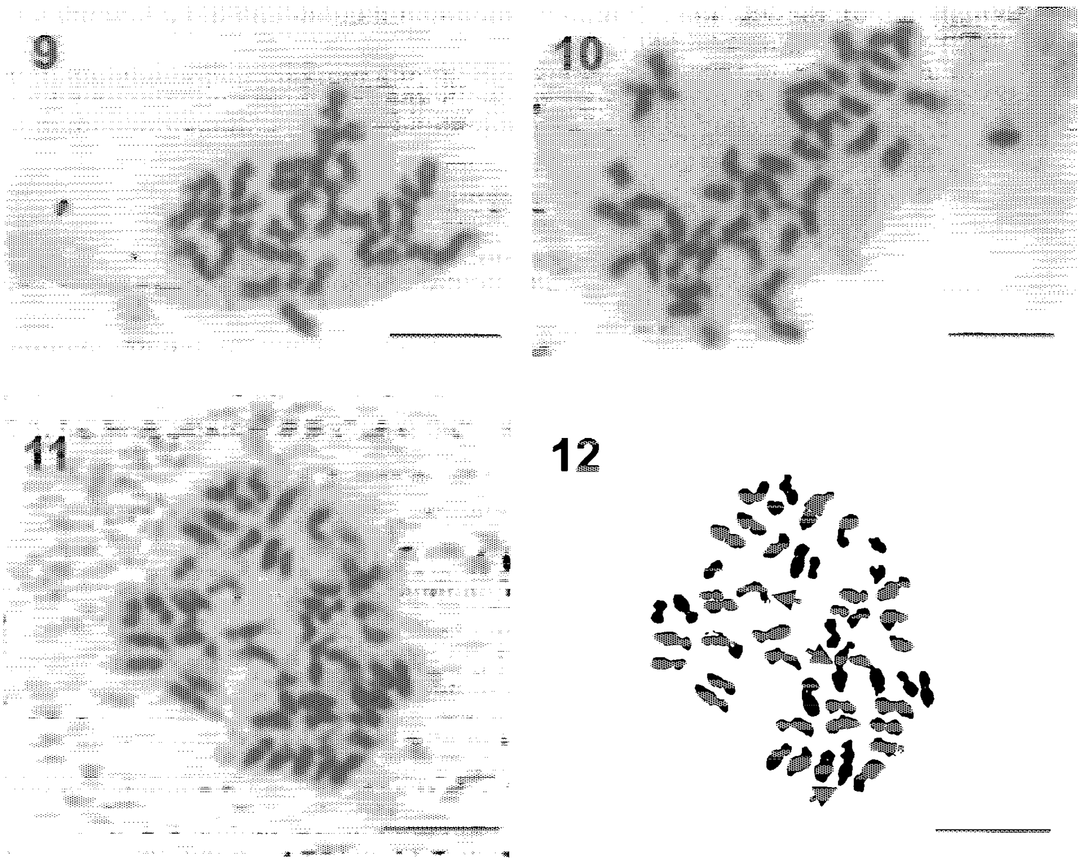
Species	Locality	2n (N)*	Voucher specimen
Sect. *Ceratanthera*			
G. campsophylla K. Schum.	Philippines	32 (3)	Takano cult. 12 (KYO)
G. franciscii Ridley	Malaysia, Sabah	32 (5)	Kato *& al.* 1365 (TI)
G. leucantha Miq.			
var. *bicolor* Holttum	Malaysia, Pahang	32 (1)	Kato *& al.* 10003 (TI)
	Indonesia, W Sumatra, HPPB**	32 (2)	Takano cult. 26 (KYO)
	Indonesia, W Sumatra, Harau Nature Reserve	32 (1)	Mori & Takano 8668 (BO)
	Indonesia, W Sumatra, Sawahlunto Sijunjung	48 (2)	Mori & Takano 9202 (BO)
var. *flavidula* Holttum	Indonesia, W Sumatra, Pasaman	32 (2)	Mori & Takano 8836 (BO)
G. maculata Blume	Indonesia, Java, Mt. Halimum National Park	48 (1)	Kato *& al.* 1149 (BO)
G. paniculata Valeton	Indonesia, W Sumatra, Ulu Gadut	32 (6)	Kato *& al.* 8609 (BO)
	Indonesia, W Sumatra, Pasaman-1	32 (2)	Mori & Takano 8802 (BO)
	Indonesia, W Sumatra, Pasaman-2	32 (2)	Mori & Takano 8846 (BO)
	Indonesia, Aceh, Mt. Leuser National Park	48 (5)	Kato *& al.* 8624 (BO)
	Indonesia, Aceh, Siongol Ongol	48 (2)	Kato *& al.* 8614 (BO)
G. pendula Roxb.	Indonesia, W Sumatra, Harau Nature Reserve	32 (3)	Mori & Takano 8665 (BO)
	Indonesia, W Sumatra, Sawahlunto Sijunjung	32 (2)	Mori & Takano 9201 (BO)
	Indonesia, N Sumatra, Bkt Lawang	32 (1)	Kato *& al.* 8657 (BO)
G. platystachya Baker	Thailand, Doi Inthanon	32 (1)	Tamura 7024 (KYO)
Sect. *Globba*			
G. acehensis A. Takano & H. Okada			
	Indonesia, Aceh, Mt. Leuser National Park	48 (3)	Kato *& al.* 8625 (BO)
G. albobracteata N. E. Br.	Indonesia, W Sumatra, Mt. Singgalang	48 (5)	Mori & Takano 8853 (BO)
G. atrosanguinea Teysm. & Binn.			
var. *atrosanguinea*	Indonesia, W Sumatra, Harau Nature Reserve	48 (2)	Mori & Takano 8666 (BO)
G. aurantiaca Miq.	Indonesia, Jambi, Sako	48 (1)	Kato *& al.* 8404 (BO)
	Indonesia, W Sumatra, HPPB**	48 (1)	Takano cult. 28 (KYO)
	Indonesia, W Sumatra, Ladang Padi	48 (3)	Kato *& al.* 8481 (BO)
	Indonesia, W Sumatra, Sipisang	48 (3)	Takano cult. 27 (KYO)
G. cernua Baker	Indonesia, Aceh, Mt. Kemiri	48 (3)	Kato *& al.* 8641 (BO)
G. fecunda A. Takano & H. Okada			
	Indonesia, W Sumatra, Lembah Anai	32 (3)	Kato *& al.* 8603 (BO)
G. flavibractea A. Takano & H. Okada			
	Indonesia, W Sumatra, HPPB**	48 (2)	Takano cult. 30 (KYO)
	Indonesia, W Sumatra, Ladang Padi	48 (4)	Kato *& al.* 8482 (BO)
	Indonesia, W Sumatra, Sipisang	48 (3)	Takano cult. 31 (KYO)
	Indonesia, W Sumatra, Ulu Gadut	48 (6)	Kato *& al.* 8490 (BO)
G. multifolia A. Takano & H. Okada			
	Indonesia, W Sumatra, Ladang Padi	32 (3)	Kato *& al.* 8481 (BO)
	Indonesia, W Sumatra, Sipisang	48 (4)	Takano cult. 29 (KYO)
	Indonesia, W Sumatra, Ulu Gadut	32 (3)	Kato *& al.* 8492 (BO)
G. patens Miq.	Indonesia, Aceh, Mt. Kemiri	48 (2)	Kato *& al.* 8636 (BO)
	Indonesia, W Sumatra, Airsirah	48 (2)	Kato *& al.* 8600 (BO)
	Indonesia, W Sumatra, Harau Nature Reserve	48 (2)	Mori & Takano 8695 (BO)
	Indonesia, W Sumatra, Pasaman	48 (1)	Mori & Takano 8824 (BO)
G. talangensis A. Takano & H. Okada			
	Indonesia, W Sumatra, Mt. Talang	48 (2)	Mori & Takano 8854 (BO)
G. sp. *1*	Malaysia, Tahan	32 (5)	Kato *& al.* 10001 (TI)

* N: number of individuals observed.

** HPPB = Study forest of Andalas University, near Padang.



FIGS. 1-8. Somatic chromosomes of *Globba*. 1-2. Microphotographs of chromosomes at metaphase of *Globba leucantha* var. *bicolor* (1. 2n=32, 2. 2n=48). 3-6. *Globba paniculata* (3-4. 2n=32, 5-6.2n=48). 3, 5. Microphotographs of chromosomes. 4, 6. Drawings of respective photographs. Arrows show satellites. 7-8. *Globba franciscii* (2n=32). 7. Microphotograph of chromosomes at metaphase. 8. Drawing of photograph. Arrows show satellites. Scale = 5 μm.



FIGS. 9-12. Somatic chromosomes of *Globba*. 9-10. Microphotographs of chromosomes at metaphase of *Globba multifolia* (9. 2n=32, 10. 2n=48). 11-12. Microphotographs of chromosomes at prometaphase of *Globba flavibracteata* (2n=48). 11. Microphotograph of chromosomes. 12. Drawing of photograph. Arrows show satellites. Scale = 5 μm.

chromosome numbers for seven species (*G. acehensis*, *G. albobracteata*, *G. aurantiaca*, *G. fecunda*, *G. flavibracteata*, *G. multifolia*, *G. talangensis*) plus the unidentified *Globba* sp. 1. I found only 2n = 48 in *G. cernua* and *G. patens*, although cytotypes of 2n = 32 and 48 have been reported (Lim 1972).

Chromosome length varied gradually from 0.7 to 3.5 μm among the species studied. In all the species the longest chromosome was not more than three times the length of the shortest one. Metacentric and submetacentric chromosomes were dominant. In *G. flavibracteata*, nine chromosomes were submetacentric and the remaining 39 were metacentric. Three metacentric chromosomes had a satellite at the distal end of the short arm (Figs. 11, 12).

Pollen fertility (Table 3)

Pollen fertility in all taxa with 2n = 32 was high (more than 85 %). Among plants with exclusively 2n = 48, *G. maculata*, *G. acehensis*, *G. aurantiaca*, and *G. talangensis* showed moderate pollen fertility (50-80 %), while the other species had lower pollen fertility (less than 50 %, Table 3). In species with cytotypes of both 2n = 32 and 48, such as *G. paniculata* and *G. leucantha* var. *bicolor*, pollen fertility of plants with 2n = 32 was high (> 90 %), while fertility in individuals with 2n = 48 was relatively low (< 75 %). In *G. multifolia*, pollen fertility was determined for plants for which the chromosome number was undetermined. One of the two populations of this species showed high pollen fertility (97±4 %), while

TABLE 3. Pollen fertility of the species of *Globba*.

Species	Locality (see Table 2)	Fertility (Mean ± S. D.) % (N)*
Taxa with 2n=32		
Sect. *Ceratanthera*		
G. campsophylla	Cultivated, collected from Philippines	95± 7 (7)
G. franciscii	Cultivated, collected from Malaysia	100± 0 (5)
G. leucantha		
var. *flavidula*	Pasaman	92± 4 (2)
	Cultivated, collected from Pasaman	98± 1 (3)
G. pendula	Harau Nature Reserve	86±14 (4)
	Cultivated, collected from Harau	90±14 (3)
G. platystachya	Cultivated, collected from Thailand	86±24 (3)
Sect. *Globba*		
G. fecunda	Cultivated, collected from Lembah Anai	99± 0 (6)
G. sp. *1*	Malaysia, Tahang	88 (1)
Taxa with 2n=32, 48		
Sect. *Ceratanthera*		
G. leucantha		
var. *bicolor*	HPPB (2n=32)	91±17 (7)
	Sawahlunto Sijiunjung (2n=48)	73±15 (10)
G. paniculata	Mt. Leuser National Park (2n=48)	61±11 (3)
	Cultivated, collected from Mt. Leuser N. P. (2n=48)	61±19 (4)
	Ulu Gadut (2n=32)	100 (1)
	Pasaman-2 (2n=32)	100± 0 (5)
	Cultivated, collected from Pasaman-2 (2n=32)	94 (1)
Sect. *Globba*		
G. multifolia	Ladang Padi (2n=32)	70±24 (6)
	Ulu Gadut (2n=32)	97± 4 (3)
Taxa with 2n=48		
Sect. *Ceratanthera*		
G. maculata	Mt. Halimun National Park	65±12 (7)
Sect. *Globba*		
G. acehensis	Mt. Leuser National Park	20±16 (3)
	Cultivated, collected from Mt. Leuser N. P.	59± 4 (2)
G. albobracteata	Harau Nature Reserve[1]	50±25 (3)
	Mt. Singgalang	20±20 (3)
G. atrosanguinea		
var. *atrosanguinea*	Harau Nature Reserve	37± 7 (5)
var. *sumatrana*	W Sumatra, Pasaman, Malampah Nature Reserve[2]	17±12 (4)
G. aurantiaca	HPPB	47±31 (2)
	Cultivated, collected from HPPB	82± 4 (2)
	Ladang Padi	65±21 (5)
	Sipisang	37±16 (5)
G. flavibracteata	HPPB	2± 2 (2)
	Ladang Padi	26± 0 (2)
	Sipisang	10 (1)
	Ulu Gadut	35±30 (6)
G. patens	Sipisang	3± 3 (6)
	Ulu Gadut	17± 8 (5)
	Cultivated, collected from Mt. Kemiri	2± 0 (2)
G. talangensis	Mt. Talang	50±15 (9)

* N: number of indiciduals observed.

[1] Chromosome number not studied. Voucher: Mori & Takano 8667 (BO).

[2] Chromosome number not studied. Voucher: Mori & Takano 8839 (BO).

the other had relatively low fertility (70±24%).

Discussion

The cytology and pollen fertility of 19 species in two sections of *Globba* were determined in this study. All the species in sects. *Ceratanthera* and *Globba* had either 2n = 32 or 48 somatic chromosomes. In some species both 2n = 32 and 48 cytotypes were observed. I concluded, for the following reasons, that plants with 2n = 32 are diploids and those with 2n = 48 are triploids with a basic chromosome number of x = 16.

The lowest somatic chromosome number in these sections is 32. Larsen (1972) examined 13 species in Thailand and presumed that plants with 2n = 16 could be found in wet tropical forests. I have examined most of the species from Sumatra and one species from Borneo (*G. franciscii*). Counts are now available for 12 of the 14 species of *Globba* in western Malaysia, 5 of 8 in Borneo, and 15 of 16 in Sumatra, none of which are 2n = 16. All counts show 2n = 32, 48 or 80. The likelihood that species with 2n = 16 can be found in the wet tropical forests is therefore greatly reduced.

In both sects. *Ceratanthera* and *Globba*, pollen fertility of taxa with 2n = 32 is high; fertility in taxa with 2n = 48 is relatively low. Lim (1972) also reported pollen fertility in plants with 2n = 48 to be greatly reduced in comparison with plants with 2n = 32. The reduced fertility in plants with 2n = 48 might be due to their triploid nature.

Plants with 2n = 32 reproduce both sexually and vegetatively, while those with 2n = 48 usually produce no fruits and propagate by rhizomes and/or bulbils on the inflorescence axis (Takano pers. observ.). Lim (1972) also mentioned that seed set has not been observed in the field in taxa with 2n = 48, nor in herbarium specimens except in *G. variabilis* (9.8% of the 121 herbarium sheets of this species in the Singapore Botanic Gardens had seeds).

Two homologous satellite chromosomes were found in some plants with 2n = 32 (*G. paniculata* and *G. franciscii*), while three were observed in plants with 2n = 48 (*G. paniculata* and *G. flavibracteata*) in this study. These features may indicate that plants with 2n = 48 are triploids. Two kinds of satellite chromosomes in plants with 2n = 48 in *G. paniculata* suggest that they may be allotriploids.

Although Lim (1972) proposed a base number of x = 8 instead of 16 for *Globba* on the basis of the occurrence of four subgroups with eight bivalents at miosis in 2n = 32 taxa and eight trivalents in 2n = 48 taxa, it is doubtful, as Newman (1988) pointed out, that such chromosome subgroups had not been reported other studies.

My study revealed many triploid species and triploid populations in Sumatra, especially in sect. *Globba*. All of the taxa are restricted to Sumatra, except *G. atrosanguinea* var. *atrosanguinea*, which also grows in Borneo. Since different species of *Globba* often grow side by side, the possibility of hybridization has already been suggested (Holttum 1950, Lim 1973). Lim (1973) demonstrated hybridization between *G. cernua* (2n = 32, 48) and *G. patens* (2n = 32, 48) based on the morphological and cytological characters. Bulbils, produced frequently on the floral axis of *Globba* in both diploids and triploids, make for efficient dispersal propagules and might ensure that propagation is unchanged in hybrids (Holttum 1950). Usually triploids are considered to have arisen from hybrids between diploid and tetraploid species. No tetraploid species are distributed in the wet tropics, however, and hybridization between tetraploids and diploids seems unlikely in Sumatra. Triploids might be derived from diploid relatives or through hybridization between allied species and fertilization between unreduced (2n) and reduced gametes (n).

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Appendix.

Notes on an unnamed *Globba* species.

Globba sp. 1. (Kato *et al.* 10001)

This species is a perennial herb to 25 cm (to the highest leaf sheath) of sect. *Globba*. It is similar to *G. corneri* and *G. unifolia* in bearing few leaves and an abruptly decurved inflorescence. Its fertile bract is pale green and not large and conspicuous as are the white bracts of *G. corneri* and the rose colored ones of *G. unifolia*. The ovary and fruit of this species are distinct in being yellowish green in contrast to the white ovaries and fruits of *G. corneri* and the orange ones of *G. unifolia*.

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